Dihydroxy Conjugates of 1,3,7,8- and 1,4,7,8-Tetrachlorodibenzo-*p*-dioxins in Rat Bile

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Introduction

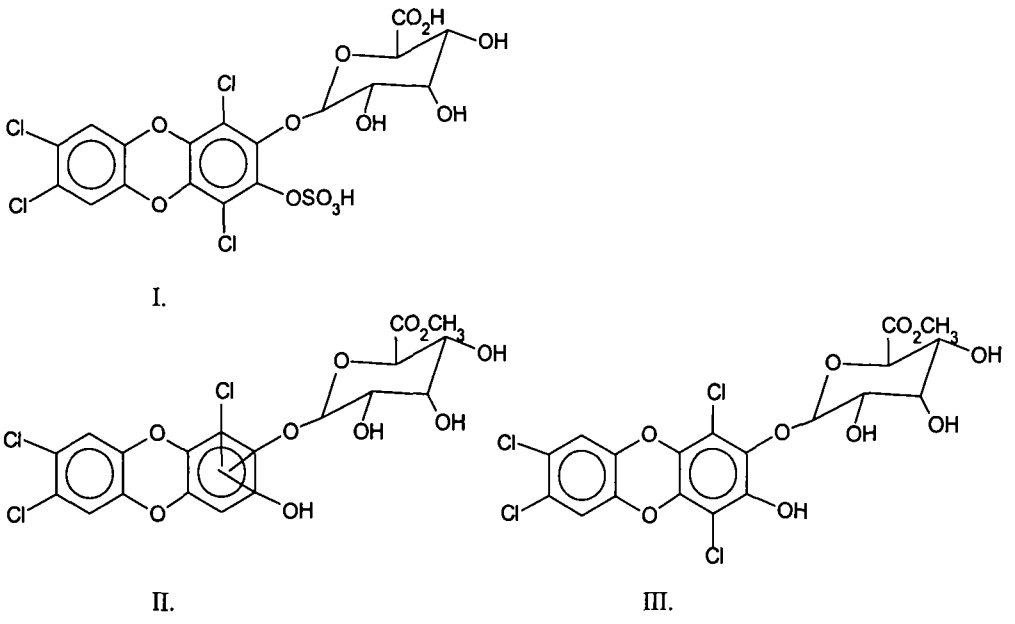
Metabolism studies of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) and other extremely toxic congeners are hindered by their slow rates of excretion and the small amounts of compound which can be administered without causing death. These factors make the isolation and identification of metabolites difficult. To alleviate these problems, we have studied the metabolism of 1378-TCDD and 1478-TCDD, two relatively nontoxic congeners, which can be dosed to rats at the mg/kg level and are almost entirely excreted within 72 h. Little work has been done on "nontoxic" dioxin congeners; although, they are present in the environmental dioxin pool to which we are exposed. Metabolism products of these congeners were shown to be similar to those reported for 2378-TCDD[1]. The major fecal and biliary metabolites in rats were previously identified as monohydroxylated TCDDs and the glucuronide conjugates, respectively, with NIH shifts occurring in the 1378-TCDD hydroxylation products[2,3]. Hydrolytic dechlorination produced a secondary metabolite of 1478-TCDD similar to one reported for 2378-TCDD. We now report the identification of dihydroxy metabolites in rat bile.

Experimental

Experimental details have been reported previously[2,3]. Male Sprague-Dawley rats were dosed orally with either ^{14}C-labelled 1378-TCDD (6 mg/kg) or ^{14}C-labelled 1478-TCDD (8 mg/kg). Bile was collected every 24 hours for three days. Biliary metabolites were purified by C_{18} HPLC methods using CH_3CN:H_2O and CH_3OH:H_2O gradients. Trifluoroacetic acid (0.1%) was added to the CH_3CN:H_2O mobile phase in some of the HPLC methods used in the 1378-TCDD experiment. Additional HPLC purification on a 4.6 x 250 mm pentafluorophenyl column (ES Industries, Berlin, NJ) with an CH_3CN:H_2O gradient was needed to resolve the dihydroxy conjugates of 1378-TCDD. Samples used to identify metabolites in this study had been partially purified and stored at -20°C for close to one year. ^{1}H-NMR spectra were acquired at 400 MHz in CD_3OD.

Results

Metabolite fractions which were more polar on reverse phase HPLC than the monohydroxy glucuronide conjugates of 1378-TCDD and 1478-TCDD were isolated from the 0-24 h bile collections. One fraction accounting for 5% of the ^{14}C in the 0-24 h bile for 1478-TCDD (2% of the total dose) was identified as a dihydroxy-TCDD conjugated to a glucuronide and a sulfate (I, Figure 1). Negative ion FAB MS showed an M-1 at 607 (four chlorines), M-81 at 527 (four



I.

II.

III.

Figure 1. Proposed structures of biliary dihydroxy conjugates of 1378-TCDD and 1478-TCDD.

chlorines), M-177 at 431 (four chlorines), and M-80-177 at 351 (four chlorines). ^{1}H-NMR showed two aromatic protons at δ = 7.23 and 7.21 ppm which supported the assigned structure.

The polar metabolite fraction from the 1378-TCDD experiment represented 20% of the ^{14}C in the 0-24 h bile (6% of the total dose) and had been tentatively identified as a sulfate glucuronide conjugate[2]. Attempts at further purification on a pentafluorophenyl column resulted in the isolation of two metabolites of approximately equal amounts. The earlier eluting peak was identified as a dihydroxy-TriCDD conjugated to a glucuronide methyl ester (II, Figure 1). Negative ion FAB MS showed an M-1 at 507 (three chlorines), M-35 at 473 (two chlorines), and M-191 at 317 (three chlorines). ^{1}H-NMR showed three aromatic protons at δ = 6.84, 7.14, and 7.19 ppm consistant with the assigned structure. The substitution pattern on the 1,4-ring is not specifically known. The other 1378-TCDD metabolite was identified as a dihydroxy-TCDD conjugated to a glucuronide methyl ester (III, Figure1). Negative ion FAB MS showed an M-1 at 541 (four chlorines), M-35 at 507 (three chlorines), and M-191 at 351 (four chlorines). ^{1}H-NMR showed one aromatic proton resonance at δ = 7.23 ppm consistant with the assigned structure where the protons on the 7,8-ring are not resolved at 400 MHz.

Both of the dihydroxy metabolites found in the bile of rats dosed with 1378-TCDD were conjugated to a glucuronide methyl ester as indicated by the loss of a 191 amu moiety in the mass spectrum. We believe that the methylation occurred as an artifact of the purification procedure due to the use of 0.1% trifluoroacetic acid in some of the early HPLC steps and the length of storage. Trifluoroacetic acid is a relatively strong acid and may have catalyzed the methylation if a suitable methyl donor were present. Sulfate groups may also have been cleaved by the acid during the workup procedure or storage. None of the dihydroxy conjugates were amenable to

enzymatic cleavage by β-glucuronidase. This may be due to steric hindrance around the site of glucuronidation or in the case of the 1378-TCDD metabolites, due to the presence of the methyl ester.

Conclusion

The metabolism of the nontoxic 1378- and 1478-TCDD congeners paralleled the metabolism reported for 2378-TCDD; however in the case of 1378- and 1478-TCDD, intact bile metabolites were isolated and identified. Indirect identification after digestion with strong acids or enzymes such as β-glucuronidase or aryl sulfatase was not required. In fact we found the dihydroxy conjugates of these dioxins to be relatively resistant to enzymatic hydrolysis which may mean their presence would be missed if relying solely on aglycone detection. The use of acid modifiers in HPLC methods and extended periods of storage appeared to result in artifact formation and probably hydrolysis of sulfate conjugates.

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